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AND BLOOD PROTEINS IN THE URINE AFTER
INTRAVENOUS INJECTION OF CRYSTALLIZED
SQUASH SEED GLOBULIN

By

CECIL CLAUDE HARVEY

B.S., University of Louisville, 1929

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AN ABSTRACT OF A THESIS

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THE EXCRETION OF SQUASH SEED GLOBULIN AND BLOOD PROTEINS IN THE URINE AFTER INTRAVENOUS INJECTION OF CRYSTALLIZED SQUASH SEED GLOBULIN*

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THE fact that the parenteral injection of foreign proteins into animals causes proteinuria has been recognized for some time. Since Berzelius' observation of this phenomenon, a number of studies of the effect of the injection of foreign protein have been reported in the literature. Inasmuch as the literature on this subject has been recently reviewed by Briggs,¹ only those papers more specifically related to the present discussion will be cited here. In summary, it may be stated that most of these previous investigations have been carried out with complex mixtures of antigenic proteins, such as egg white and blood sera. The excretion of proteins was tested for, in almost all cases, by means of chemical tests which are not sufficiently delicate to detect very small traces of protein, and are not, except in rare instances,[†] capable of identifying the protein. It seemed desirable, therefore, to study the elimination of a pure protein and to investigate at the same time the excretion of blood proteins following the injection of the foreign protein. Two such studies have been carried out in this laboratory, one using crystallized ovalbumin, the other using crystallized squash seed globulin for injection. The two studies were carried out for the most part simultaneously and, as far as possible, by the use of similar techniques. The excretion of proteins in the urine of injected dogs was followed by testing for the injected protein and for dog serum albumin and pseudoglobulin by means of the precipitin reaction.

The results of the investigation using crystallized ovalbumin have already been reported by Briggs.¹ The results of experiments with crystallized squash seed globulin are reported in this paper. This protein had not previously been used in this type of study. Two somewhat similar proteins, edestin and excelsin, were used by Mendel and Rockwood,³ who reported that they were not excreted in unchanged form. The tests used by these investigators, however, were not capable of detecting very small traces of protein.

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†In some cases the protein used for injection allowed more specific deductions to be made. Thus Pavy,^{2a} after the injection of isinglass, detected both gelatin and coagulable protein in the urine. Parisot and his co-workers^{2b} were able to show the excretion of globin and blood proteins following the injection of globin.

PREPARATION OF MATERIAL

The proteins used were prepared in as pure a form as possible. Crystallized squash seed globulin was prepared according to Osborne's method,⁴ and purified by four recrystallizations. The blood proteins were prepared by Welker's method.⁵ All antisera for these proteins were obtained by intramuscular injection of rabbits with the individual proteins adsorbed on aluminum hydroxide, according to the method of Hektoen and Welker.⁶ Each antiserum had a titer of at least 1:100,000, and was specific for the antigen used in its preparation.

PRECIPITIN TESTS

All precipitin tests were made by the contact layer method. A drop of the solution to be tested in progressive dilutions was layered over a drop of antiserum, and the highest dilution of the substance which would give a white band precipitate at the point of contact after standing for one hour at room temperature was determined.

IMMEDIATE EFFECT OF A FOREIGN PROTEIN ON THE KIDNEY

In order to determine the immediate effect of a foreign protein on the kidney of the dog, three medium-sized dogs were injected with crystallized globulin, and urine specimens were collected for an hour. This was accomplished by inserting a No. 8 hard rubber catheter into the bladder of a male dog, and after a preliminary period of ten to twenty minutes, introducing 10 c.c. of a 1 per cent solution of crystallized squash seed globulin in 6 to 7 per cent sodium chloride solution into the lesser saphenous vein. The urine excreted over short time intervals was collected and tested by the precipitin reaction for the presence of crystallized squash seed globulin, dog serum pseudoglobulin, and dog serum albumin. In all three experiments the urine before the injection of the foreign protein gave negative tests for both dog blood proteins. The results of these experiments are summarized in Table I.

TABLE I

TIME OF INITIAL APPEARANCE OF PROTEINS IN THE URINE FOLLOWING THE INJECTION OF CRYSTALLIZED SQUASH SEED GLOBULIN

DOG	WEIGHT IN KG.	MINUTES AFTER INJECTION OF SQUASH SEED GLOBULIN BEFORE APPEARANCE OF		
		CRYSTALLIZED SQUASH SEED GLOBULIN	SERUM PSEUDOGLOBULIN	SERUM ALBUMIN
31	8.2	7	7	14
32	6.4	10	10	15
33	6.8	5	5	5

Effect of Repeated Injections of a Pure Foreign Protein on the Elimination of the Foreign Protein and the Blood Proteins.—Male dogs, weighing 6 to 14 kg., were used as experimental animals. During the period of injection the dogs were kept in metabolism cages on a complete diet, and the urine was collected under toluene. Each series of injections was preceded by a control period varying from ten to eighty days. During the control period the animals were

kept under experimental conditions, and occasional samples of urine were collected and examined for the presence of dog serum pseudoglobulin and dog serum albumin.

One per cent solutions of crystallized squash seed globulin dissolved in 6 to 7 per cent sodium chloride solution were injected into the leg veins. The injections were repeated within two to six days, so that the physiologic action would take place in an immune, rather than a sensitive, animal. After the completion of the series of injections, the dogs were kept in stock pens, or cages, and returned to the metabolism cages occasionally for periods of observation. The urine specimens were filtered and tested by the precipitin reaction for the presence of crystallized squash seed globulin, dog serum pseudoglobulin, and dog serum albumin.

Ten adult male dogs and one female puppy were given 4 to 35 injections of crystallized squash seed globulin. The duration of the injection period was determined by the effect of the solution injected on the veins of the dog's legs. In some animals the solution caused rapid occlusion of the vessels; in others this action appeared more slowly. The animals may be divided into the following groups:

Group 1.—Dogs 1, 2, 3, 5, and 10 received from 64 to 152 c.c. of 1 per cent squash seed globulin in from 6 to 13 doses, extending over periods of from thirteen to ninety-eight days. Dog 4 received 528 c.c. of this foreign protein solution in 35 injections during a period of one hundred and five days.

Group 2.—Dogs 7, 8, 9, and 11 received from 10 to 72 c.c. of the foreign protein solution in from 10 to 20 doses, extending over periods of from fifty-seven to one hundred days.

Group 3.—Dog 6 received 202 c.c. of 1 per cent squash seed globulin solution in 4 injections over a period of ten days.

TABLE II
REPEATED INJECTION OF CRYSTALLIZED SQUASH SEED GLOBULIN

DOG	WEIGHT IN KG.	NO. OF INJECTIONS	AMOUNT INJECTED IN GM.	DURATION OF INJECTION PERIOD IN DAYS	DURATION OF OBSERVATION PERIOD AFTER LAST INJECTION IN DAYS
1	11.0	8	0.79	24	1,555
2	7.7	6	1.52	21	2
3	11.3	7	1.44	13	7
4	11.3	35	5.28	105	33
5	9.1	6	0.64	14	7
10	10.0	13	1.05	98	52
7	13.6	10	0.38	57	336
8	10.9	20	0.72	113	
9	5.6	5	0.10	21	Died
11	9.1	11	0.51	85	57
6	9.3	4	2.02	10	Died

Table II shows the amount of squash seed globulin injected into each animal, the duration of the injection period, and the length of the period of observation following the last injection.

EXCRETION OF SQUASH SEED GLOBULIN

In the case of 5 of the 6 dogs of group 1, analysis of the urine excreted during the injection period showed that about 35 per cent of the urine samples contained squash seed globulin. In the case of Dog 10, however, only 3 of 57 specimens gave a positive test for the protein injected. With the dogs of group 2, which received smaller doses of foreign protein, a smaller fraction (about 15 per cent) of the urine excreted during the injection period gave positive reactions for the protein injected. One dog of this group, Dog 11, showed little tendency to excrete squash seed globulin, which appeared in but 3 of 48 samples. These results are summarized in Table III.

TABLE III
EXCRETION OF SQUASH SEED GLOBULIN

DOG	NUMBER OF TESTS MADE*	POSITIVE TESTS WITH DIFFERENT DILUTIONS OF URINE					TOTAL NUMBER OF POSITIVE TESTS	PER-CENTAGE OF TESTS POSITIVE
		UNDILUTED	1:10	1:100	1:1,000	1:10,000		
1	54	10	4	4	1	2	21	39
2	26	6	2	1	0	0	9	35
3	17	4	0	1	0	0	5	29
4	104	36	1	1	0	0	38	35
5	18	7	0	0	0	0	7	39
10	57	0	0	1	0	2	3	5
7	53	9	0	0	0	0	9	17
8	97	12	2	0	0	0	14	14
9	18	3	1	0	0	0	4	22
11	48	0	0	0	0	3	3	6
6	8	3	2	0	0	0	5	63

*These figures include urine excreted for seven days following the last injection, except in the following cases: Dogs 6 and 9 died following the last injection, and only one urine sample was obtained after the final injection. Dog 2 died two days after the final injection.

In the animals of group 1 the excretion of squash seed globulin decreases after the first 3 or 4 injections. This tendency is shown in Table IV. Opie⁷

TABLE IV
PER CENT OF URINE SPECIMENS GIVING POSITIVE REACTIONS FOR SQUASH SEED GLOBULIN AT DIFFERENT STAGES OF THE INJECTION PERIOD

DOG	DURING THE FIRST PART OF INJECTION PERIOD	DURING THE LAST PART OF INJECTION PERIOD
1	50	27
3	44	13
4	55	27
5	55	22

has reported similar findings with ovalbumin. He expressed the belief that as immunization proceeds the injected animal acquires an increased ability to get rid of the injected protein. Some of Briggs' findings¹ are in accord with Opie's opinion.

After the final injection of crystallized squash seed globulin, the elimination of this protein practically ceases after two to four days. Occasional samples, however, show the presence of the foreign protein for a period of eight to twenty-one days after the final injection. Dog 1 was observed, one

TABLE V
EXCRETION OF BLOOD PROTEINS

DOG	BEFORE INJECTION			AFTER INJECTION							PER CENT OF POSITIVE TESTS IN HIGHER DILUTIONS
	NO. OF TESTS MADE	POSITIVE TESTS		NO. OF TESTS MADE	POSITIVE TESTS		NO. OF POSITIVE TESTS IN HIGHER DILUTIONS				
		NO.	%		NO.	%	1:10	1:100	1:1,000		
<i>Pseudoglobulin</i>											
1	5	0	0	131	124	95	69	5	0	56	
2	6	3	50	25	25	100	15	0	0	60	
3	5	5	100	17	17	100	9	2	0	65	
4	6	1	17	140	103	74	8	1	0	6	
5	6	3	50	19	15	79	0	0	0	0	
10	20	15	80	77	69	90	18	0	0	23	
7	26	9	35	58	53	91	10	0	0	17	
8	54	17	32	102	88	85	9	0	0	9	
9	13	10	77	18	8	44	1	2	0	17	
11	14	5	36	74	58	78	11	0	0	15	
6	15	14	93	8	7	88	3	1	0	50	
<i>Albumin</i>											
1	5	0	0	131	124	95	41	2	1	34	
2	6	2	33	25	25	100	17	0	0	67	
3	5	5	100	17	17	100	10	2	0	71	
4	6	4	67	140	131	94	16	1	0	12	
5	6	6	100	19	19	100	1	2	0	5	
10	20	20	100	77	73	95	18	0	0	26	
7	26	5	19	58	32	55	6	0	0	10	
8	54	15	28	102	63	62	5	2	0	7	
9	13	5	38	18	8	44	3	1	0	22	
11	14	5	36	74	69	93	16	0	0	22	
6	15	15	100	8	8	100	5	0	1	75	

year after the final injection, to resume the excretion of small traces of squash seed globulin in several urine samples over a period of twenty-three days (see Table VI).

The amount of squash seed globulin excreted represents only a small part of that injected. The major portion must be either stored in the tissues, metabolized in the body, or excreted in a form which does not react with the specific antiserum. Such an immunologically altered form might result from the combination of the foreign protein with blood proteins. Welker, Andrews, and Thomas⁸ have suggested that albuminuria is a result of a protective mechanism in which toxic substances are combined with blood proteins with the formation of a foreign protein which is excreted. It was not possible in this investigation to show that crystallized squash seed globulin formed such combinations.

Hunter⁹ has expressed the opinion that the nonexcreted fraction of parenterally injected foreign proteins is digested by proteolytic enzymes elaborated by leucocytes. This hypothesis is based on his observation that during the immunization of rabbits with ox serum and ox serum proteins, each injection was followed by a leucocytosis which increased with each injection.

EXCRETION OF BLOOD PROTEINS

Before injection the urine of most of the dogs gave occasional tests for one or both of the blood proteins. Such positive tests were obtained only on

TABLE VI

EXCRETION OF SQUASH SEED GLOBULIN AND BLOOD PROTEINS FOLLOWING INTRAVENOUS INJECTION OF CRYSTALLIZED SQUASH SEED GLOBULIN (DOG 1)

TIME AFTER INITIAL INJECTION IN HOURS	AMOUNT OF 1% CRYST. SQUASH SEED GLOBULIN INJECTED IN C.C.	PRECIPITIN REACTIONS OF URINE WITH ANTISERUM FOR		
		CRYST. SQUASH SEED GLOBULIN	SERUM PSEUDO- GLOBULIN	SERUM ALBUMIN
Control		0	0	0
	5			
5		4	2	2
22		5	1	1
24		3	1	1
45		1	1	1
72		0	1	1
96		0	0	1
124		0	1	1
147		0	1	0
171				
	5			
173		0	2	2
189		2	2	1
193		5	3	1
216		2	2	0
241		0	2	1
262		1	2	2
286		0	0	1
288				
	10			
291		1	1	1
294		3	3	1
309		2	1	2
314		0	3	1
334		3	2	1
339		1	2	1
340		0	2	2
362				
	15			
364		0	2	2
381		1	1	1
405		1	0	1
429		0	2	1
436		0	1	1
457				
	9			
459		0	2	3
460		1	2	2
476		1	3	4
478				
	17			
480		1	1	1
481		0	2	2
500		0	2	2
523		0	2	1
526				
	5			
527		0	2	1
548		0	1	2
561		3	2	1
595		0	1	1
598				
	13			
602		0	1	1
620		0	2	1
626		1	2	2
644		1	2	2
668		0	1	1
692		0	2	3
706		0	2	1
763		0	2	1
768		0	1	1
812		0	2	1
836		0	2	2
860		0	2	2
884		3	2	2
911		0	2	2
958		0	2	2
1,006		1	1	2

TABLE VI—CONT'D

TIME AFTER INITIAL INJECTION IN DAYS	AMOUNT OF 1% CRYST. SQUASH SEED GLOBULIN INJECTED IN C.C.	PRECIPITIN REACTIONS OF URINE WITH ANTISERUM FOR		
		CRYST. SQUASH SEED GLOBULIN	SERUM PSEUDO- GLOBULIN	SERUM ALBUMIN
43		0	1	2
45		2	0	1
46		0	0	0
49		0	0	0
50		0	0	1
51		0	1	2
52		0	1	2
53		0	1	1
220		0	2	2
221		0	1	1
222		0	1	1
385		0	1	1
388		0	1	2
390		0	2	1
391		2	2	2
391		1	2	1
394		0	2	1
395		0	2	2
396		0	2	2
397		0	2	1
398		1	1	1
399		0	2	1
400		0	0	0
401		0	2	1
403		0	1	1
404		0	2	1
405		1	2	1
407		1	2	1
408		1	2	2
409		0	2	2
410		1	2	1
412		1	2	1
414		1	1	1
415		0	2	1
416		0	1	1
425		0	2	1
426		0	2	1
428		0	1	1
429		0	1	1
431		0	1	1
432		0	2	1
434		0	1	1
435		1	1	1
436		0	1	1
437		0	1	1
438		0	1	1
774		0	2	1
775		0	1	1
776		0	2	1
781		0	1	1
782		0	2	2
785		0	2	2
1,172		0	2	2
1,173		0	2	1
1,174		0	2	2
1,574		0	2	2
1,575		0	2	2
1,576		0	1	1
1,577		0	3	0
1,578		0	2	2

0 = No reaction.

1 = Reaction in undiluted urine.

2 = Reaction in all specimens through the 1:10 dilution.

3 = Reaction in all specimens through the 1:100 dilution.

4 = Reaction in all specimens through the 1:1,000 dilution.

5 = Reaction in all specimens through the 1:10,000 dilution.

the undiluted urine, never, except with Dog 6, with urine that had been diluted with 9 or more parts of saline. Immediately after the injection of squash seed globulin, the excretion of the blood proteins was increased. This increased excretion can be noted in the greatly increased frequency with which the urine gives positive tests for these proteins, and by the fact that a number of samples after injection give positive tests when diluted with 9 parts of saline; occasional samples give positive tests when diluted with 99 or more parts of saline. The increase in the excretion of blood proteins was observed in all the dogs tested. The findings are listed in Table V. This increase in the excretion of blood proteins continues after the final injection of squash seed globulin has been given and the foreign protein no longer appears in the urine.

The excretion of foreign protein was always accompanied by the elimination of one or both blood proteins. Two different dogs on one occasion excreted hemoglobin as well as the two plasma proteins. These urines did not contain red blood cells nor did they hemolyze dog erythrocytes. Squash seed globulin was present in one of these hemoglobin-containing urines but not in the other.

The excretion of blood proteins continues long after the urine has ceased to contain squash seed globulin. The excretion of these proteins cannot be correlated with any known factor. Samples usually contained both blood proteins in equal amounts; at times the amount of pseudoglobulin was greater than that of albumin, at other times it was less. Some urine specimens contained only one of the serum proteins, and occasional samples were protein free. The ratio in which these proteins were excreted was not related either to the difference in their molecular size nor to the difference in their concentration in the blood. The process, therefore, cannot be considered as one of simple filtration.

Table VI gives the results of the urine analyses of Dog 1. The results are, for the most part, typical of those obtained with the animals of group 1.

The amounts of protein found in these urines were too small to be detected by the usual chemical tests and would not be considered clinically as pathologic. On the occasions when hemoglobin was excreted, the condition was only transitory and cleared up within a few hours. The histologic examination of sections of liver, spleen, and kidney showed no changes which could be attributed to the experimental procedure. These results are similar to those obtained by Briggs¹ in her study with ovalbumin. Brull¹⁰ has found that dogs can excrete egg white for weeks without presenting a subsequent tendency to true proteinuria.

SUMMARY

The injection of crystallized squash seed globulin into the blood of medium-sized dogs was followed by its appearance in the urine within five to ten minutes. It continues to be excreted for a period of twenty-four to seventy-two hours.

Following repeated injections, the amount of squash seed globulin excreted tends to decrease. Some foreign protein is excreted at irregular intervals after the last injection.

The amount of squash seed globulin excreted represents only a small portion of that injected. The larger portion could not be accounted for.

The appearance of squash seed globulin in the urine was always accompanied by either serum albumin or serum pseudoglobulin, or both. Repeated injection of squash seed globulin did not cause an increase in the amount of serum proteins excreted.

The injection of crystallized squash seed globulin into medium-sized dogs caused neither immediate kidney damage nor had a cumulative effect on the kidney.

I wish to express my appreciation to Dr. William H. Welker for his advice in the conduct of this investigation and to Dr. George Milles for his kindness and cooperation in examining the histologic material.

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